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The Role of the Brain-Derived Neurotrophic Factor (*BDNF*) *val66met* Variant in the Phenotypic Expression of Obsessive-Compulsive Disorder (OCD)

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Evidence suggests that the *Val66Met* variant of the brain-derived neurotrophic factor (*BDNF*) gene may play a role in the etiology of Obsessive-Compulsive Disorder (OCD). In this study, the role of the *BDNF Val66Met* variant in the etiology and the phenotypic expression of OCD is investigated. Associations between the *BDNF Val66Met* variant and OCD, obsessive-compulsive symptom dimensions, Yale-Brown Obsessive Compulsive Scale (YBOCS) severity scores, age of onset and family history of obsessive-compulsive symptoms were assessed. The *BDNF Val66Met* variant was genotyped in 419 patients with sub-/clinical OCD and 650 controls. No differences in allele or genotype frequency were observed between cases and controls. In females with OCD, the *Met66Met* genotype was associated with later age of onset and a trend for a negative family history, whereas the *Val66Val* genotype was associated with a trend for lower YBOCS severity scores. Item-level factor analysis revealed six factors: 1) Contamination/cleaning; 2) Aggressive obsessions/checking; 3) Symmetry obsessions, counting, ordering and repeating; 4) Sexual/religious obsessions; 5) Hoarding and 6) Somatic obsessions/checking. A trend was found for a positive association between Factor 4 (Sexual/religious obsessions) and the *BDNF Val66Val* genotype. The results suggest that *BDNF* function may be implicated in the mediation of OCD. We found that for the *BDNF Met66Met* genotype may be associated with a milder phenotype in females and a possible role for the *BDNF Val66Val* genotype and the *BDNF Val66* allele in the sexual/religious obsessions. © 2009 Wiley-Liss, Inc.

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INTRODUCTION

Obsessive-compulsive disorder (OCD) is both clinically and genetically heterogeneous. This heterogeneity obscures the findings of clinical, natural, and treatment response studies. Family and twin studies indicate that genetic factors play an important role in mediating at least some forms of OCD [Pauls and Alsobrook, 1999; Hettema et al., 2001; Nestadt et al., 2002; van Grootheest et al., 2005]. Although major advances have been made in the last decade in characterizing the phenomenology and psychobiology of OCD, its phenotypic and genetic heterogeneity has complicated the search for vulnerability genes.

One approach to deal with this heterogeneity is to classify OCD according to clinically defined characteristics and to search for the genetic underpinnings of these possibly more homogeneous phenotypes. Most studies that focused on the phenomenology of obsessive-compulsive (OC) symptoms used the Yale-Brown Obsessive-Compulsive Scale symptom checklist (YBOCS-CL), a screening instrument for the presence of frequently encountered OC symptoms [Goodman et al., 1989a,b]. Factor analytic studies using predefined symptom categories of the YBOCS-CL have consistently found four OC symptom dimensions, namely (1) aggressive, sexual, somatic and religious obsessions with checking obsessions; (2) symmetry obsessions with ordering and arranging compulsions; (3) contamination obsessions and cleaning compulsions, and (4) hoarding obsessions and compulsions [Miguel et al., 2005]. These symptom dimensions are hypothesized to represent more homogeneous genetic dimensions of OCD [Miguel et al., 2005]. The YBOCS-CL symptom dimension of aggressive/sexual obsessions and checking compulsions, and the symptom dimension of symmetry obsessions and ordering and arranging compulsions have been found to be more familial than the other symptom dimensions [Alsobrook et al., 1999]. A recent family study showed statistically significant correlations of scores on all symptom dimensions between affected sib pairs [Hasler et al., 2007]. Moreover, a segregation analysis of the symptom dimensions in siblings with Tourette syndrome (TS), a putative OCD spectrum disorder, suggested a familial component for the symmetry, ordering and counting factor as well as the aggression/sexual obsessions factor [Leckman et al., 2003]. Furthermore, two recent genome scans in which hoarding was used as a phenotype found suggestive linkage to regions on chromosomes 14, 4q, 5q, and 17q [Zhang et al., 2002; Samuels et al., 2007]. A recent candidate gene study recently found the "obsessional/checking" OCD symptom subtype to be associated with earlier age of OCD onset and the *Met158Met* genotype of the *COMT Val158Met* polymorphism [Lochner et al., 2008]. Three studies investigated the relation between symptom dimensions and an insertion/deletion polymorphism in the serotonin transporter gene (*5HTTP*) [Cavallini et al., 2002; Kim et al., 2005; Hasler et al., 2006]. Two of these studies found a trend for a positive association between a symptom dimension with counting and repeating rituals [Cavallini et al., 2002; Hasler et al., 2006]. The third study found an association between this polymorphism and a factor with religious and somatic obsessions [Kim et al., 2007]. Two other studies found no association between symptom dimensions and the *Val66Met* polymorphism in the Brain Derived Neurotrophic factor gene (*BDNF*) [Wendland et al., 2007; Alonso et al., 2008]. Taken together, these studies suggest that OC symptom dimensions comprise distinct genetic entities, which would justify using them as phenotypes in molecular genetic studies of OCD. Since the symptom dimensions obtained by factor analysis can be quantified as factor scores for each patient, these symptoms dimensions are statistically more powerful than the investigation of OCD as a dichotomous diagnostic entity [Silverman and Palmer, 2000].

The *BDNF* gene is an attractive OCD candidate gene, from both a brain development and neurotransmitter perspective. *BDNF* has been implicated in neuronal survival and in activity-dependent neuroplasticity [Hennigan et al., 2007]. Studies in knockout mice as well as those using B cell lines suggest that *BDNF* modulates the serotonin transporter function [Mossner et al., 2000; Daws et al., 2007].

A polymorphism in the *BDNF* gene that causes a valine to methionine substitution in the prodomain of the BDNF protein (*Val66Met*) has been shown to reduce activity-dependent BDNF secretion in transfected neurons [Egan et al., 2003]. Studies in *BDNF Met66Met* mice suggest that this polymorphism may be implicated in anxiety-related behaviors [Chen et al., 2006]. Pharmacological and neurobiological studies suggest that the serotonergic system is involved in the pathogenesis of OCD [Westenberg et al., 2007]. In summary, there are several lines of evidence indicating that the serotonergic system and, more specifically *BDNF*, may be implicated in the etiology of OCD.

Thus far, seven studies have reported on the *BDNF Val66Met* variant in OCD, using either a family based approach [Hall et al., 2003; Mossner et al., 2005; Zai et al., 2005; Dickel et al., 2007], or a case–control design [Wendland et al., 2007; Alonso et al., 2008; Hemmings et al., 2008]. These studies are summarized in Table I. Findings have been inconsistent. In the family based studies, an *under* transmission of the *BDNF Met66* allele was found in one study of patients with childhood-onset OCD [Hall et al., 2003], whereas the other studies did not show a preferential transmission of either of the alleles. In the case–control studies an association was found of the *BDNF Met66* allele with OCD in males [Hemmings et al., 2008], whereas Wendland et al. [2007] did not find an association. One family based study using haplotype analysis found a haplotype marked by the *BDNF Met66* allele to be undertransmitted and therefore likely to confer a protective effect against OCD [Hall et al., 2003], whereas another family based study found a haplotype including the *BDNF Val66* allele to be associated with a reduced risk for OCD [Alonso et al., 2008]. Some studies also investigated the association of the *BDNF Val66Met* genotype and dimensional OC phenotypes such as symptom dimensions, age of onset and symptom severity [Hall et al., 2003; Zai et al., 2005; Wendland et al., 2007; Alonso et al., 2008]. One study found an association of the *BDNF Met66* allele with earlier age of onset in males and the *BDNF Val66Met* genotype with increased severity in women [Hemmings et al., 2008], whereas other studies did not find an association with these dimensional phenotypes. Similarly, a family based study of the *BDNF Val66Met* in patients with TS (with or without comorbid OCD), a disorder genetically related to OCD, found no association between TS and the *BDNF Val66Met* polymorphism [Klaffke et al., 2006].

An important drawback of the studies mentioned concerns the relatively small sample sizes, which possibly explains the inconsistent findings to date. In this study, trying to overcome this disad-

TABLE I. Association Studies Investigating the *BDNF Val66Met* Variant in Obsessive-Compulsive Disorder and Tourette Syndrome

						Transmitted alleles		
Study	**Population**	**n**	**Diagnostic criteria**	**Diagnosis**	**Test**	**Val66**	**Met66**	***P*-value**
Family-based studies								
Hall et al. [2003] [a]	USA	164	DSM-IV	OCD	TDT	58	26	0.0005
Mossner et al. [2005] [a]	German	67	DSM-IV	OCD without TS	TDT	17	20	0.62
Zai et al. [2005] [a]	Canada	152	DSM-IV	OCD	FBAT			0.587
Dickel et al. [2007] [b]	USA	54	DSM-III-R	OCD ± TS	TDT	15	15	1.00
Klaffke et al. [2006] [a]	German	88	DSM-III-R	TS ± OCD	ETDT	24	30	0.414

							Genotype counts			*P*-values	
Study	**Population**	**n**	**Diagnostic criteria**	**Diagnosis**	**Test**		**Val/Val**	**Val/Met**	**Met/Met**	**Allelic**	**Genotypic**
Case–control studies											
Wendland et al. [2007]	Caucasian	295	DSM-IV	OCD ± tics	x^2/exact						
						Patients	192	92	11		
						Controls	428	206	23	0.950	1.000
						Male patients	82	37	5		
						Male controls	193	98	13	0.715	0.876
						Female patients	110	55	6		
						Female controls	235	108	10	0.612	0.844
Hemmings et al. [2008] [c]	Afrikaner	112	DSM-IV	OCD ± tics	x^2	Patients	73	33	6		
						Controls	95	43	2	0.355	0.249
						Male patients	33	19	5		
						Male controls	25	8	0	0.036	0.117
						Female patients	40	14	1		
						Female controls	70	35	2	0.438	0.717
Alonso et al. [2008] [b]	Spanish	115	DSM-IV	OCD ± tics	Logistic regression under dominant, recessive, additive, codominant and overdominant model					n.s.	n.s.

OCD, Obsessive-compulsive disorder; TS, Tourette syndrome; TDT, Transmission Disequilibrium Test; FBAT, Family Based Association Test; ETDT, Extended Transmission Disequilibrium Test.
[a]Data on analyses stratified by sex are not provided.
[b]Results of analysis after stratification by sex were insignificant.
[c]Part of the subjects in this study are included in the current analyses.

vantage, we investigated the *BDNF Val66Met* polymorphism in a large group of patients with OCD. The aims of our study were: (1) to replicate the association of the *BDNF Val66Met* polymorphism and OCD in patients with early-onset OCD and/or males described previously [Hall et al., 2003; Hemmings et al., 2008] (2) to investigate the association of this polymorphism with specific OC symptom dimensions, age of onset of OC symptoms (irrespective of the nature thereof), OC symptom severity and family history of OC symptoms. Since sex differences have been described in both clinical and genetic studies of OCD [Lochner et al., 2004; Labad et al., 2008], we also investigated sex-specific associations of this polymorphism with clinical characteristics of OCD.

METHODS

This project encompasses a joint venture between the Department of Psychiatry of VU University Medical Center (VUMC) and the Department of Psychiatry of the University Medical Center Groningen (UMCG) in the Netherlands and the MRC Research Unit on Anxiety Disorders, in collaboration with the MRC/US Centre for Molecular and Cellular Biology, University of Stellenbosch in South Africa. The study was approved by the Medical Ethical Review Boards of the participating centers. All patients (and in case of minors, their parents) gave written informed consent for participation in the study.

Participants

Patients at the MRC Unit on Anxiety and Stress disorders of the University of Stellenbosch were recruited by physician referral, media advertisements, the Mental Health Information Centre (MHIC) and the OCD Association of South Africa (OCDSA), as described previously [Hemmings et al., 2008]. Patients at the outpatient Clinic for Anxiety Disorders, GGZ Buitenamstel in Amsterdam and the University Medical Center in Groningen (The Netherlands) were recruited by physician referral, media advertisements and patient societies for patients with anxiety disorders.

All patients met criteria for either a lifetime (Netherlands) or a current (South Africa) diagnosis of OCD or subclinical OCD according to DSM-IV criteria [American Psychiatric Association, 1994]. Diagnoses were established using the Structured Clinical Interview for Axis I disorders (SCID-I/P) [First et al., 1998] or the Mini International Neuropsychiatric Interview (MINI) version 5.0.0. [Sheehan et al., 1998]. Subclinical OCD was defined as OC symptoms that are either time-consuming (i.e., take 1 hr a day or more, but without causing distress) or interference or that are distressing or causing interference, but take less than 1 hr a day. Only Caucasian patients and controls from the South African site were included; patients from the Netherlands were from the general Dutch population. Control subjects from both South Africa and the Netherlands encompassed an unscreened convenience sample recruited from the general population. The study included 606 phenotyped patients of whom 419 (n = 220 from The Netherlands, n = 199 from South Africa) were also genotyped for the *BDNF Val66Met* polymorphism. YBOCS-CL data were available for 579 patients. Further, genotype data were available of 650 controls (n = 535 Dutch, n = 115 Caucasian South African). Some of the subjects from the South African cohort were included in a previous case–control study [Hemmings et al., 2008].

Measurements

The YBOCS-CL [Goodman et al., 1989a,b] was used to assess OC symptom characteristics. In some patients from Amsterdam (n = 111) an extended 80-item self-response version of the YBOCS was used. This 80-item self-report YBOCS-CL was translated from the version used in the TSA genetica consortium on Tourette's disorder and within the scope of the OCF international collaboration on the genetics of OCD. In a comparison of an interview- versus self-report version of the YBOCS-CL in the US, the self-report version showed good internal consistency and test-retest reliability and strong convergent validity with the interview version [Steketee et al., 1996].

Current severity of the obsessive-compulsive symptoms was assessed using the YBOCS Severity Scale (YBOCS-SS) [Goodman et al., 1989a,b]. In contrast to YBOCS-symptom checklist data, we used the original interview-based severity scale as the YBOCS severity scale. In addition, age of onset of OC symptoms and family history of OC symptoms were determined. Family history was considered to be positive if the proband reported the presence of recognizable OC symptomatology that bothered the person in at least one first-degree family member.

Genotyping

DNA was isolated from blood using a chloroform/isopropanol extraction [Meulenbelt et al., 1995], from buccal swabs using a salting out procedure [Miller et al., 1988] or from sputum using an Oragene DNA self collection kit (DNA Genotek, Inc., Ottawa, Canada) according to manufacturers instructions.

All DNA samples from the South African cohort were obtained from blood. Genotyping of the South African cohort was performed by allele-specific restriction enzyme digestion of a PCR product with *Nla*III, as described previously [Hemmings et al., 2008]. Complete digestion was taken to be the presence of the consecutive 57 bp band.

Dutch samples were genotyped in a SNPlex™ genotyping assay (Applied Biosystems, Foster City, CA) or by Taqman genotyping assay (Applied Biosystems) (Assay on demand, ID CD_11592758_10) according to manufacturer's instructions.

All controls from the Dutch cohort were genotyped in a SNPlex™ genotyping assay: 104 blood samples with a 100% success rate and 450 buccal swab samples with 95.7% success rate. The genotypes of the Dutch patients were determined in two SNPlex™ runs with a mean success rate of 90.2% and a Taqman run with a success rate of 94.7%.

Statistical Analysis

Data from the South African and Dutch cohorts were combined. Power calculations were performed with the genetic power calculator [Purcell et al., 2003] assuming a disease prevalence of 2% [Angst et al., 2004; Weissman et al., 1994]. Hardy–Weinberg equilibrium was tested using chi square tests using Microsoft Office Excel. Genotype and allele frequencies were compared between

patients and controls and between patients with different phenotypes using Fisher's exact tests. Since data on continuous variables such as age of onset of OC symptoms, YBOCS-SS score and factor scores showed a skewed distribution, differences in these variables between patients with different genotypes and between the *BDNF Val66* and the *Met66* allele were examined using non-parametric tests (Kruskal–Wallis and Mann–Whitney *U* tests).

Factor Analyses

Different versions of the YBOCS-CL were used with some non-overlapping items. Only items overlapping with the 74-item YBOCS-CL version described by Goodman et al. [1989a,b] were used in the analysis. The extended 80-item self-report version of YBOCS-CL used for some Dutch patients, contained several items concerning obsessions with symmetry and exactness. However, since no distinction was made with respect to magical thinking accompanying these obsessions, one item was created coding for any symmetry obsession in the patients. Moreover, in the 80-item self report version of the YBOCS-CL seven items were not available (as indicated in Table IV). Missing values in the YBOCS-CL and YBOCS-SS were imputed using Solas™ 3.2 (Statistical Solutions, Ltd, Cork, Ireland), using predictive model-based imputation. For the YBOCS-CL only the items with missing values were included in the imputation model whereas for the YBOCS-SS all items were used in the imputation model. Five different datasets were imputed according to recommendations by Schafer [1999]. Items from the "miscellaneous" or "other" categories were omitted from the analysis since these symptoms were considered to be too heterogeneous.

Explorative item-by-item level factor analysis with promax rotation was performed using MPlus [Muthén and Muthén, 1998–2006]. Promax rotation was used, since this form of rotation allows the factors to be correlated. Subsequently, confirmatory factor analysis for categorical variables was performed to establish the number of factors and factor constitution using the following fit indices: the x^2 statistic, the comparative fit index (CFI), the Tucker-Lewis Index (TLI), the Root Mean Square Error of Approximation (RMSEA) and the Standardized Root Mean Square Residual (SRMR). Values of the CFI and of TLI approaching 0.95, values approaching 0.08 of the SRMR and values of the RMSEA < 0.05 are generally indicative of a good fit [Browne and Cudek, 1993; Hu and Bentler, 1999].

For the best fitting model, mean score per item for each of the obtained factors was calculated for each patient, representing the prominence of this factor in the patient. Mean results for the five imputed datasets are reported. Kruskal–Wallis tests and Mann–Whitney *U* tests were performed to investigate whether mean item score per factor were associated with the *BDNF Val66-Met* genotype or alleles. Kruskal–Wallis tests and Mann–Whitney *U* tests were performed using R statistical package [R Development Core Team, 2008]. For these tests empirical *P* values were calculated based on 1000 random permutations. This was done for the entire sample and for male and female patients separately in order to obtain robust *P*-values for each gender. Ten phenotypes were tested (OCD as a dichotomous trait, age of onset of OC symptoms, YBOCS severity score, family history and 6 symptom dimensions). These phenotypes were tested for the entire sample and for males and females separately. Because the total sample is not independent from the two genders, a *P*-value of $0.05/20 = 0.0025$ was considered significant for these tests. All statistical tests were performed using the Software Package for Social Sciences (SPSS) version 14.0 (SPSS, Inc, Chicago, IL) except noted otherwise.

Sample Size and Power Considerations

Since large samples produce more stable variable loadings across repeated sampling and more precise estimates of population loadings [MacCallum et al., 1999], we included non-genotyped participants in the factor analysis as well.

Similarly, we included previously studied Caucasian subjects from South Africa in our current association study to increase sample size and power. Based on the allele frequency in our combined control population, the power to detect a dominant

TABLE II. Demographic and Clinical Characteristics of the OCD Patients in the Different Cohorts

	Total cohort	South African cohort	Dutch cohort	*P*
All patients				
n	606	199	407	
Age (y, mean ± SD)	35.4 ± 12.7	32.8 ± 14.5	36.7 ± 11.6	<0.001
Female (%)	334 (55.1%)	95 (47.7%)	239 (58.7%)	0.011
Age of onset (y, mean ± SD)	17.4 ± 9.9	17.2 ± 10.9 (n = 188)	17.5 ± 9.3 (n = 366)	0.134
YBOCS severity (mean ± SD)	20.5 ± 8.1	19.9 ± 7.6 (n = 195)	20.7 ± 8.3 (n = 392)	0.137
Positive family history of OC behavior (%)			94 (44.3%) (n = 212)	
Genotyped patients				
n	419	199	220	
Age (y, mean ± SD)	35.3 ± 13.4	32.8 ± 14.5	37.5 ± 11.9	<0.001
Female (%)	219 (52.3%)	95 (47.7%)	124 (56.4%)	0.079
Age of onset (y, mean ± SD)	17.0 ± 10.2	17.2 ± 10.9 (n = 188)	16.7 ± 9.5 (n = 192)	0.648
YBOCS severity (mean ± SD)	20.0 ± 7.9	19.9 ± 7.6 (n = 195)	20.0 ± 8.2	0.659
Positive family history of OC behavior (%)			64 (50.8) (n = 126)	

TABLE III. Genotype and Allele Frequencies*

	Genotypes				*P*-value (Fischer's exact test)	Alleles		*P*-value (Fischer's exact test)
	Val66Val	*Val66Met*	*Met66Met*	Total		*Val66*	*Met 66*	
Total cohort								
OCD	260 (62.1%)	137 (32.7%)	22 (5.3%)	419		657 (78.4%)	181 (21.6%)	
Control	428 (65.8%)	202 (31.1%)	20 (3.1%)	650	P = 0.149	1058 (81.4%)	242 (18.6%)	P = 0.095
Male OCD	125 (62.5%)	65 (32.5%)	10 (5.0%)	200		315 (78.8%)	85 (21.3%)	
Male control	183 (65.8%)	89 (32.0%)	6 (2.2%)	278	P = 0.234	455 (81.8%)	101 (18.2%)	P = 0.247
Female OCD	135 (61.6%)	72 (32.9%)	12 (5.5%)	219		342 (78.1%)	96 (21.9%)	
Female control	245 (65.9%)	113 (30.4%)	14 (3.8%)	372	P = 0.436	603 (81.0%)	141 (19.0%)	P = 0.229
South African cohort								
OCD	128 (64.3%)	62 (31.2%)	9 (4.5%)	199		318 (79.9%)	80 (20.1%)	
Control	76 (66.1%)	36 (31.3%)	3 (2.6%)	115	P = 0.784	188 (81.7%)	42 (18.3%)	P = 0.602
Male OCD	63 (60.6%)	34 (32.7%)	7 (6.7%)	104		160 (76.9%)	48 (23.1%)	
Male control	21 (72.4%)	8 (27.6%)	0 (0.0%)	29	P = 0.350	50 (86.2%)	8 (13.8%)	P = 0.147
Female OCD	65 (68.4%)	28 (29.5%)	2 (2.1%)	95		158 (83.2%)	32 (16.8%)	
Female control	55 (64.0%)	28 (32.6%)	3 (3.5%)	86	P = 0.730	138 (80.2%)	34 (19.8%)	P = 0.498
Dutch cohort								
OCD	132 (60.0%)	75 (34.1%)	13 (5.9%)	220		339 (77.0%)	101 (23.0%)	
Control	352 (65.8%)	166 (31.0%)	17 (3.2%)	535	P = 0.123	870 (81.3%)	200 (18.7%)	P = 0.065
Male OCD	62 (64.6%)	31 (32.3%)	3 (3.1%)	96		155 (80.7%)	37 (19.3%)	
Male control	162 (65.1%)	81 (32.5%)	6 (2.4%)	249	P = 0.887	405 (81.3%)	93 (18.7%)	P = 0.914
Female OCD	70 (56.5%)	44 (35.5%)	10 (8.1%)	124		184 (74.2%)	64 (25.8%)	
Female control	190 (66.4%)	85 (29.7%)	11 (3.8%)	286	P = 0.071	465 (81.3%)	107 (18.7%)	P = **0.025**

*P values <0.0025 were considered significant; P values below the nominal significance level of 0.05 are indicated in bold.

effect of the *BDNF Met66* allele with a two times increased risk for OCD in the *BDNF Val66Met* and *Met66Met* genotypes compared to the *Val66Val* genotype in the case-control study was 99.9%, 92.0%, and 95.8% in the total sample and males and females separately, respectively. The power to detect a recessive effect of the *BNDF Met66* allele with a two times increased risk for OCD with the *BDNF Met66Met* genotype compared to the *BDNF Val66Met* and *Val66Val* genotypes was 34.0% in the total sample, 17.3% in males, and 21.6% in females.

RESULTS

Demographic and clinical characteristics of the patients studied are summarized in Table II. The Dutch patients were significantly older than the South African patients ($P < 0.001$). The Dutch phenotyped group contained significantly more women ($P < 0.012$) than the South African group, although this difference did not reach significance in the genotyped patients.

Case–Control Study

Genotype and allele frequencies, as well as case–control study results are summarized in Table III. Genotype distribution was in Hardy–Weinberg equilibrium for all groups. There was no significant difference in genotype ($P = 1.000$) or allele frequency ($P = 0.926$) between the South African and Dutch control groups, nor were there differences in genotype or allele frequency between male and female controls from South Africa and from the Nether-

TABLE IV. Goodness of Fit Indices Obtained by Confirmatory Factor Analysis*

Fit index	1 factor model	2-factor model	3-factor model	4-factor model	5-factor model	6-factor model
CFI	0.506	0.715	0.845	0.853	0.881	0.889
TLI	0.650	0.802	0.891	0.897	0.916	0.920
RMSEA	0.110	0.083	0.062	0.060	0.054	0.053
SRMR	0.177	0.144	0.119	0.117	0.111	0.112

CFI, Comparative fit index; TLI, Tucker-Lewis Index; RMSEA, Root Mean Square Error of Approximation; SRMR, Standardized Root Mean Square Residual.
*Values of the CFI and of TLI approaching 0.95, values approaching 0.08 of the SRMR and values of the RMSEA < 0.05 are generally indicative of a good fit.

TABLE V. Mean Factor Loadings for the Five Imputed Datasets Obtained by Confirmatory Factor Analysis*

Factor 1: Contamination and cleaning	
Concern with dirt or germs	1.000
Excessive or ritualized hand washing[a]	0.851
Concerns or disgust with bodily waste or secretion (e.g., urine, faeces, and saliva)	0.772
Excessive concern with household items (e.g., cleaners, solvents)[a]	0.758
Excessive concern with environmental contaminants (e.g., asbestos, radiation, toxic waste)	0.743
Bothered by sticky substances or residues	0.712
Compulsions involving cleaning of household items or other inanimate objects	0.705
Other measures to prevent or remove contact with contaminants	0.696
Excessive or ritualized showering, bathing, teeth brushing, grooming, or toilet routine	0.684
Excessive concern with animals (e.g., insects)	0.676
Concerned that I will get ill because of contaminant	0.481
Concerned that I will get others ill by spreading contamination (aggressive)[a]	0.466
No concern with consequences of contamination other than how it might feel[a]	0.390
Factor 2: Aggressive obsessions and checking	
Fear that I will harm others because of not being careful enough (e.g., hit/run MVA)	1.000
Fear that I will steal things	0.738
Fear that I might harm others	0.720
Checking that I did not/will not harm others	0.707
Fear that I will act on unwanted impulses (e.g., to stab a friend)	0.696
Fear that I will be responsible for something else terrible happening (e.g., fire, burglary)	0.691
Fear of blurting out obscenities or insults	0.694
Checking that nothing terrible did/will happen	0.655
Fear of doing something else embarrassing	0.606
Fear that I might harm myself	0.537
Violent or horrific images	0.408
Checking that I did not/will not harm self	0.430
Checking that I did not make a mistake	0.428
Concerned that I will get others ill by spreading contamination (aggressive)[a]	0.361
Checking locks, stove, appliances, etc.	0.359
Factor 3: Symmetry obsessions, ordering, arranging, counting, and repeating compulsions	
Ordering/arranging compulsions	1.000
Re-reading or re-writing	0.685
Counting compulsions	0.643
Symmetry obsessions	0.634
Need to repeat routine activities (e.g., in/out door, up/down from chair, i.e., repeating rituals)	0.537
Checking that I did not make a mistake	0.478
Checking locks, stove, appliances, etc.	0.293
Factor 4: Sexual an religious obsessions and compulsions	
Forbidden or perverse sexual thoughts/images/impulses	1.000
Content (of obsession) involves children or incest[a]	0.842
Content (of obsession) involves homosexuality[a]	0.841
(Obsession with) sexual behavior toward others (aggressive)[a]	0.763
Excessive concern with right/wrong, morality	0.747
Concerned with sacrilege and blasphemy	0.691
Violent or horrific images	0.323
Factor 5: Hoarding obsessions and compulsions	
Hoarding compulsions	1.000
Hoarding obsessions	0.931
Factor 6: Somatic obsessions and checking	
Concern with illness or disease	1.000
Checking tied to somatic obsessions	0.711
Excessive concern with a body part or an aspect of appearance (e.g., dysmorphophobia)	0.620
Concerned that I will get ill because of contaminant	0.430
Checking that I did not/will not harm self	0.466

*Items shown in italic show high loadings on more than one factor.
[a]Item missing in the extended 80-item self report version.

TABLE VI. Mean Cronbach's Alpha for the Different Factors and Perason's Correlations for the Mean Score Per Item Between the Different Factors

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Cronbach's alpha	0.844	0.786	0.688	0.669	0.829	0.630
Factor 2	0.339	1.000				
Factor 3	0.263	0.421	1.000			
Factor 4	0.156	0.466	0.148	1.000		
Factor 5	0.143	0.197	0.219	0.128	1.000	
Factor 6	0.500	0.416	0.199	0.225	0.126	1.000

lands respectively (genotypes: $P = 0.834$ in males, and $P = 0.920$ in males; alleles: $P = 0.472$ in males, $P = 0.740$ in females). Therefore, these groups were pooled for the subsequent analyses. There was a trend for an association between the *BDNF Met66* allele and OCD in female Dutch patients ($P = 0.025$). This was not found in the South African sample or in the pooled sample.

Factor Analysis

YBOCS-CL data were available of 579 OCD patients. In the exploratory factor analysis, thirteen factors with an eigenvalue > 1 were identified. Forty-three YBOCS-CL items were included in the factor analysis, with the number of participants being higher than the recommended 5–10 subjects per item. Based on the results from the iterative exploratory factor analyses, we performed confirmatory factor analyses to obtain a more parsimonious model. The fit indices of the one to six factor models are summarized in IV. The one to six factor models had an increasingly better fit. Since the seven factor model did not converge, models with 7 or more factors were not investigated.

The six factor model showed the best fit and fulfilled the criteria for satisfactory model fit and was therefore used in subsequent analyses. Factor loadings for this model are shown in Table V. This model had the following factors:

Factor 1: Contamination obsessions and cleaning compulsions; Factor 2: Aggressive obsessions and checking; Factor 3: Symmetry obsessions, ordering, arranging, counting, and repeating compulsions; Factor 4: Sexual and religious obsessions; Factor 5: Hoarding obsessions and compulsions; and Factor 6: Somatic obsessions and checking.

Pearson's correlations between mean scores per item for the different factors and Cronbach's alpha for internal consistency of the different factors are summarized in Table VI. Although mean scores per item for the different factors were significantly correlated (data not shown), the correlations between them were relatively low.

Genotype–Phenotype Correlations

Both genotype data and mean scores per item for the different factors were available of 392 patients with OCD. The results of the Kruskal–Wallis and Mann–Whitney *U* tests comparing mean scores per item for the different factors between patients with different genotypes are summarized in Table VII. None of the factors showed an association with *BDNF Val66Met* genotype after correction for multiple-testing. However, there was a trend for a positive association between factor 4 (Sexual and religious obsessions) and the *BDNF Val66Val* genotype in the patients.

The results of the comparisons of age of onset of OC symptoms and OCD severity by *BDNF Val66Met* genotype are summarized in Table VIII. There was a significant sex effect with respect to age of onset of OC symptoms and genotype; age of onset of OC symptoms in women with the *BDNF Met66Met* genotype was significantly later than in the other groups ($P = 0.002$). Further, there was a trend towards lower OCD severity in female patients with the *BDNF Val66Met* or *Met66Met* genotypes compared to female patients with the *BDNF Val66Val* genotype ($P = 0.023$).

There was no difference in genotype ($P = 0.577$) or allele frequency ($P = 0.282$) between patients with and patients without a positive family history of OC symptoms. However, none of the women with the *BNDF Met66Met* genotype had a family history of OC symptoms compared to 36.1% of the patients with other genotypes ($P = 0.019$).

DISCUSSION

In this study, the *BDNF Val66Met* polymorphism was investigated in a large group of patients with OCD. Female patients with the *BDNF Met66Met* genotype had a later age of onset of OC symptoms and there was a trend for a lower OCD severity in female patients with one or more *BDNF Met66* alleles. Moreover, female patients with a negative family history of OC symptoms more often had the *BDNF Met66Met* genotype. Although these results should be considered preliminary, given the low number of female patients with the *BDNF Met66Met* genotype ($n = 12$) and that data on age of onset of OC symptoms were collected retrospectively, the findings suggest that the *BDNF Met66* alleles may have a protective role in females with OCD. The finding of a protective effect of the *BDNF 66Met* allele is in line with a previous study (including patients of both sexes) that found the *BDNF 66Met* alleles to be *under*-transmitted in early onset OCD cases [Hall et al., 2003]. Early onset seems to be linked to more severe OCD [Sobin et al., 2000; Fontenelle et al., 2003]. Other studies in which no association was found between the *BDNF Val66Met* variant and age of onset [Zai et al., 2005; Wendland et al., 2007] had the drawback that smaller

TABLE VII. Kruskal–Wallis and Mann–Whitney *U* Tests Comparing Scores Per Item for the Different Factors Between Patients With Different *BDNF Val66Met* Genotype*

	Val66Val	*Val66Met*	*Met66Met*	*P*-value	*Val66Met* + *Met66Met*	*P*-value	*Val66Val* + *Val66Met*	*P*-value	*Met66*	*Val66*	*P*-value
All patients											
n	246	125	21								
Mean factor 1 items	0.231 ± 0.249	0.187 ± 0.227	0.245 ± 0.236	0.190	0.195 ± 0.229	0.178	0.216 ± 0.242	0.494	0.201 ± 0.229	0.222 ± 0.245	0.400
Mean factor 2 items	0.237 ± 0.218	0.219 ± 0.186	0.197 ± 0.186	0.801	0.216 ± 0.185	0.736	0.231 ± 0.208	0.551	0.213 ± 0.185	0.233 ± 0.212	0.603
Mean factor 3 items	0.423 ± 0.292	0.440 ± 0.300	0.367 ± 0.200	0.530	0.430 ± 0.288	0.703	0.429 ± 0.294	0.388	0.422 ± 0.279	0.426 ± 0.293	0.981
Mean factor 4 items	0.143 ± 0.205	0.112 ± 0.187	0.082 ± 0.178	0.057	0.107 ± 0.185	**0.046**	0.133 ± 0.200	0.086	0.104 ± 0.184	0.137 ± 0.202	**0.017**
Mean factor 5 items	0.199 ± 0.374	0.188 ± 0.363	0.143 ± 0.359	0.728	0.182 ± 0.361	0.651	0.195 ± 0.370	0.393	0.177 ± 0.360	0.197 ± 0.371	0.507
Mean factor 6 items	0.187 ± 0.251	0.147 ± 0.228	0.152 ± 0.178	0.299	0.147 ± 0.221	0.190	0.173 ± 0.244	0.814	0.148 ± 0.216	0.179 ± 0.247	0.311
Males											
n	119	59	9								
Mean factor 1 items	0.204 ± 0.246	0.182 ± 0.220	0.248 ± 0.210	0.536	0.190 ± 0.218	0.832	0.197 ± 0.237	0.324	0.197 ± 0.217	0.200 ± 0.240	0.852
Mean factor 2 items	0.245 ± 0.238	0.228 ± 0.203	0.133 ± 0.129	0.452	0.216 ± 0.197	0.759	0.240 ± 0.226	0.215	0.206 ± 0.191	0.242 ± 0.230	0.466
Mean factor 3 items	0.403 ± 0.300	0.441 ± 0.304	0.397 ± 0.234	0.755	0.435 ± 0.295	0.486	0.416 ± 0.301	0.843	0.430 ± 0.287	0.411 ± 0.300	0.644
Mean factor 4 items	0.171 ± 0.226	0.140 ± 0.209	0.143 ± 0.247	0.546	0.140 ± 0.212	0.310	0.161 ± 0.220	0.476	0.141 ± 0.215	0.165 ± 0.222	0.249
Mean factor 5 items	0.168 ± 0.346	0.212 ± 0.385	0.222 ± 0.441	0.792	0.213 ± 0.389	0.507	0.183 ± 0.359	0.867	0.214 ± 0.393	0.177 ± 0.353	0.538
Mean factor 6 items	0.200 ± 0.269	0.168 ± 0.251	0.111 ± 0.145	0.734	0.161 ± 0.240	0.444	0.189 ± 0.263	0.669	0.155 ± 0.230	0.194 ± 0.265	0.417
Females											
n	127	66	12								
Mean factor 1 items	0.255 ± 0.250	0.191 ± 0.235	0.242 ± 0.263	0.171	0.199 ± 0.238	0.081	0.525 ± 0.764	0.963	0.205 ± 0.241	0.242 ± 0.248	0.127
Mean factor 2 items	0.229 ± 0.199	0.210 ± 0.171	0.244 ± 0.212	0.894	0.216 ± 0.176	0.915	0.222 ± 0.190	0.705	0.219 ± 0.180	0.225 ± 0.193	0.942
Mean factor 3 items	0.441 ± 0.285	0.439 ± 0.298	0.345 ± 0.177	0.497	0.425 ± 0.284	0.892	0.440 ± 0.288	0.251	0.414 ± 0.273	0.441 ± 0.286	0.560
Mean factor 4 items	0.117 ± 0.181	0.087 ± 0.162	0.036 ± 0.089	0.111	0.079 ± 0.154	0.060	0.107 ± 0.175	0.127	0.073 ± 0.147	0.111 ± 0.177	**0.034**
Mean factor 5 items	0.228 ± 0.397	0.167 ± 0.343	0.083 ± 0.289	0.295	0.154 ± 0.335	0.221	0.207 ± 0.380	0.192	0.144 ± 0.329	0.216 ± 0.386	0.109
Mean factor 6 items	0.175 ± 0.232	0.127 ± 0.206	0.183 ± 0.199	0.271	0.136 ± 0.205	0.294	0.159 ± 0.224	0.428	0.142 ± 0.204	0.165 ± 0.227	0.539

*P values <0.0025 were considered significant; P values below the nominal significance level of 0.05 are indicated in bold.

TABLE VIII. Kruskal–Wallis and Mann–Whitney *U* and Fisher's Exact Tests Comparing YBOCSS Scores, Age of OC Symptom Onset and Family History Between Patients With Different *BDNF Val66Met* Genotype*

	Val66Val	n	Val66Met	n	Met66Met	n	*P*	Val66Met + Met/Met	*P*	Val66Val + Val66Met	*P*	Val66	Met 66	*P*
YBOCS severity score														
All patients	20.3 ± 7.8	248	19.5 ± 7.7	130	20.3 ± 9.1	22	0.667	19.6 ± 7.9	0.453	20.0 ± 7.8	0.885	20.1 ± 7.8	19.7 ± 8.0	0.550
Females	21.4 ± 7.8	130	18.7 ± 7.9	70	20.0 ± 9.6	12	0.051	18.9 ± 8.1	**0.023**	20.5 ± 7.9	0.991	20.8 ± 7.8	19.1 ± 8.3	0.063
Males	19.0 ± 7.7	118	20.5 ± 7.4	60	20.6 ± 9	10	0.450	20.5 ± 7.6	0.217	19.5 ± 7.6	0.805	19.3 ± 7.6	20.5 ± 7.7	0.239
Age of onset														
All patients	17.2 ± 10.1	235	15.9 ± 9.6	126	21.7 ± 13.8	19	0.135	16.6 ± 10.4	0.415	16.7 ± 9.9	0.127	16.9 ± 10.0	17.2 ± 10.9	0.937
Females	17.1 ± 10.6	120	15.9 ± 9.7	68	26.9 ± 12.2	11	**0.008**	17.4 ± 10.7	0.843	16.7 ± 10.3	**0.002***	16.9 ± 10.4	18.6 ± 11.2	0.192
Males	17.2 ± 9.6	115	15.8 ± 9.6	58	14.5 ± 13.2	8	0.296	15.7 ± 10	0.167	16.7 ± 9.6	0.215	16.9 ± 9.6	15.5 ± 10.3	0.104
Positive family history														
All patients	31	53	15	28	2	6	0.577	17	0.510	46	0.401	92	19	0.282
Females	22	31	9	16	0	4	**0.022**	9	0.083	15	**0.019**	53	9	**0.009**
Males	9	22	6	12	2	2	0.310	8	0.495	31	0.216	24	10	0.256

*P values <0.0025 were considered significant; P values reaching statistical significance are indicated with an asterisk; P values below the nominal significance level of 0.05 are indicated in bold.

samples were used which might have hampered power to detect between-group differences.

Little is known about a sex-specific association between the *BDNF Val66Met* polymorphism, OCD symptom severity and age of onset of OC symptoms. To our knowledge, the only other study that has directly investigated this, found an association between the *BDNF Met66* allele and an earlier age of onset of OCD in males [Hemmings et al., 2008]. In apparent contrast, our findings suggested a significant association between the *BDNF Met66Met* genotype in females and later age of OC symptom onset, coupled with a trend towards lower OC symptom severity. Our findings may be analogous to animal studies that have suggested sex-dependent differences in heterozygous $BDNF^{+/-}$ knockout mice, with female heterozygous *BDNF*/homozygous serotonin transporter gene (*5HTT*)-knockout mice showing less increase in anxiety-like behaviors as well as less reduction of serotonin concentrations in several brain regions than males [Ren-Patterson et al., 2006]. These findings suggest that female sex may protect against reduced *BDNF* function in mice, at least in the presence of *5HTT*-deficiency. The relationship between sex and serotonin levels might result from differences in hormone status between men and women. In estrogen-receptor β-knockout mice decreased concentrations of serotonin and dopamine have been found, combined with increased anxiety reactions, independent of current estradiol suppletion [Imwalle et al., 2005]. Moreover, it has been shown that estrogen increased expression of tryptophan hydroxylase 2 (TPH2), the rate limiting enzyme in serotonin synthesis, in the caudal region of the raphe nuclei of ovariectomized rats and that expression of TPH2 mRNA in this region is correlated with anxiety-like behavior [Hiroi et al., 2006]. Therefore, sex dependent effects on anxiety symptoms might be the result of a serotonin enhancing effect of estrogen at the level of the caudal raphe nuclei. Recently, increasing evidence suggest that changes in the glutamatergic system may be implicated in OCD [Pittenger et al., 2006]. BDNF has been shown to influence the glutamatergic system by alteration of the composition and plasticity of glutamatergic synapses [Carvalho et al., 2008]. Therefore, the effects of the *BDNF Val66Met* polymorphism may also be mediated by the glutamatergic pathway.

There are several lines of evidence in support of our findings indicating that age of onset of OC symptoms and OCD symptom severity are important aspects of the OCD phenotype that may have specific genotypic underpinnings. Previous studies suggest that age of onset in OCD has two distributions [Delorme et al., 2005]. Moreover, early onset OCD has been found to represent a more familial phenotype of the disorder [Lenane et al., 1990; Pauls et al., 1995; Delorme et al., 2005].

Item-level factor analysis of the YBOCS-CL identified six OCD symptom dimensions. The contamination/cleaning, symmetry/ordering, and hoarding symptom dimensions were similar to the factors consistently found in previous category-based factor analyses. The aggressive obsession/checking factor often found in category-based factor analysis usually comprises aggressive, sexual, and religious obsessions and in some studies this factor also included somatic obsessions. In our item-level factor analysis, these symptoms seemed to be divided over three factors: aggressive obsessions/checking, sexual and religious obsessions and somatic obsessions and related checking.

We found a trend for higher mean score per item for Factor 4 (sexual and religious obsessions) in patients with the *BDNF Val66Val* genotype than in patients with other genotypes. This may suggest a protective effect for the *BDNF Met66* alleles against sexual and religious obsessions. This finding has to be considered with caution however, since a considerable amount of missing data were imputed for items in this factor. Two previous studies found that the *BDNF Val66Met* polymorphism was not significantly associated with any OCD symptom dimension [Wendland et al., 2007; Alonso et al., 2008]. However, these studies comprised smaller sample sizes, category-based analyses and only one study specifically investigated the sexual and religious obsession symptom category [Alonso et al., 2008].

A limitation of our study is the limited power to detect recessive effects of the *BDNF Met66* allele. Since the allele frequency of the *BDNF Met66* allele is low, larger sample sizes are needed to detect recessive effects. Only the *Val66Met* polymorphism was investigated, which is a limitation of this study. To our knowledge, the *Val66Met* polymorphism is the only known functional polymorphism in the *BDNF* gene. Therefore, we thought it was justified to focus on this polymorphism only. A limitation of case–control association studies is that these can be biased by population stratification [Thomas and Witte, 2002]. Family-based studies have been developed to circumvent this problem [Schulze and McMahon, 2002]. Alternatively, multiple markers can be typed to test and correct for possible population stratification by genomic control or structured association methods [Pritchard and Donnelly, 2001]. The use of these methods in future studies would be desirable.

Further, gene–gene interactions that might confer increased risk for OCD or dimensional OCD phenotypes were not investigated.

To our knowledge, only two studies investigated gene-gene interaction involving the *BDNF* gene both with negative results. Both studies have a relatively limited sample size, which may have caused their negative results. The first study investigating interaction between the *BDNF Val66Met* polymorphism and the *5HTT* gene promoter polymorphism found no evidence for a gene-gene interaction in OCD [Wendland et al., 2007]. However, the negative result of this study might have been the consequence of not taking into account the effect of negative life events on the interaction between the *BDNF Val66Met* and the *5HTTPR* polymorphism, as found in studies in depression [Kaufman et al., 2006; Kim et al., 2007]. The second study investigated the interaction between the *BDNF* gene and the gene encoding its specific receptor, the neurotrophic tyrosine kinase 2 gene (*NTRK2*) gene [Alonso et al., 2008]. In summary, studies investigating the role of gene-gene interaction of the *BDNF Val66Met* polymorphism and other genes as well as gene x environment interactions in the etiology of OCD are warranted.

In conclusion, our results suggest that in females, the *BDNF Met66Met* genotype may be associated with a late-onset form of OCD and the *BDNF Val66Val* genotype with a more severe form of OCD. Future studies including relevant variables such as sex, age of onset, severity of OC symptoms and OC symptom dimensions obtained by item level factor analysis in a sample large enough to detect recessive effects of the *BDNF Val66Met* polymorphism are needed to replicate and extend our current findings.

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